International Immunology, Vol. 25, No. 6, pp. 345–352
doi:10.1093/intimm/dxs156
Advance Access publication 4 February 2013

Phosphocholine-binding antibody activities are hierarchically encoded in the sequence of the heavy-chain variable region: dominance of self-association activity in the T15 idiotype

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Received 20 October 2012, *accepted* 13 December 2012

Abstract

A methodology based on the representation of each amino acid of a protein sequence by the electron-ion interaction potential and subsequent analysis by signal processing was used to determine the characteristic or common frequency (in Hz) that reflects the biological activity shared among phosphocholine (PC)-binding antibodies. The common frequency for the variable portion of the heavy chain (VH) of the PC-specific antibodies is found to be at *f* = 0.37 Hz. The VH sequences of the PC-binding antibodies exhibit three subsites for the PC moiety where hypervariable region 2 (CDR2) plays a role in the interaction with the phosphate group. Mutations in this VH region have an impact on the ability of mutant variants to bind PC and its carrier molecule, as well as on the characteristic frequency shift toward *f* = 0.12 Hz for mutants failing to bind both hapten and carrier. The VH sequence of mutants that retain the ability to bind PC still shows *f* = 0.37 Hz, suggesting that this frequency determines PC binding. However, this statement was not confirmed as mutation in another PC subsite impairs PC binding but retains both the phosphate-group recognition and the frequency at *f* = 0.37 Hz. Herein, this finding is discussed to promote the idea that the VH sequence of the PC-binding antibodies encodes the subsite for phosphate-group binding as a dominant functional activity and that only CDR2 of the T15-idiotype antibodies together with FR3 region form an autonomous self-association function represented by the T15VH50–73 peptide with *f* = 0.37 ± 0.05 Hz. Thus, these data confirmed that T15VH50-73 peptide might be used in superantibody technology.

Keywords: antibodies with autophilic properties, informational spectrum method, resonant recognition model, superantibodies

Introduction

The sequence diversity of antibodies results from the combinatorial re-arrangement of multiple germline variable (V), diversity (D) and joining (J) genes but additional diversity is generated through junction diversity, as well as subsequent somatic mutation of the already re-arranged genes (1). The somatic diversification of expressed V region genes that occurs during the immune response to phosphocholine (PC) is a biologically important problem because of the frequent expression of this haptenic epitope on complex antigens such as the cell wall and some capsular polysaccharides (PS) of pathogenic bacteria, as well as on apoptotic cell membranes and oxidized low-density lipoproteins (2, 3). Thus, the need for maintenance of genes for antibodies against PC in the germline or relatively unchanged configuration in different mice strains is logical. In agreement with this belief is the finding that immune response in mice to the PC moiety is highly restricted as an equivalent set of antibodies to PC in genetically dissimilar mice is maintained, implying that genes encoding these antibodies are likewise quite similar or in some cases identical (4). The anti-PC antibodies raised to PC antigens comprise three main families that were initially based on differential recognition by anti-idiotypic (anti-Id) antibodies. One family shares public Id determinants found on a group of nearly identical PC-binding myeloma proteins (MP) S107/TEPC-15 (also termed the T15 idiotype family) and two others on anti-PC MP, MOPC-167/511 (also termed M511) and McPC-603 (also termed M603) (5, 6).

High doses of Id antibodies directed against anti-PC antibodies have been shown to lead to clonal deletion of T15-bearing B cells (7). These idiotype families are very closely related and their heavy-chain variable region (VH) domains are transcribed from the same VH gene segment (6, 7). However, each of the three families uses a distinct V region for its light (L) chains encoded by three different VL genes, VK22, VK24 and VK8, respectively (8–11). The presence of PC-binding antibody in the idiotype families T15, M603 and M511 was described in studies on hybridoma proteins generated from different strains of mice immunized with *Streptococcus pneumoniae* (*S. pneumoniae*) (R36a) and PC protein (12).

The T15 VH gene family in BALB/c mice includes three functional gene segments one of which, the V1 gene segment, encodes virtually all VH domains in BALB/c anti-PC antibodies (9, 13, 14). In other mouse strains, the T15 VH gene element can be matched with its allelic counterpart in BALB/c mice (15). The T15 idiotype-positive antibodies dominate the response of BALB/c mice to pneumococcal C polysaccharides and are important in the protection of mice from experimental pneumococcal infection (16). Thus, the primary antibody response after immunization of BALB/c mice with PC-containing antigens, either associated with bacterial polysaccharides or coupled to protein carriers, is dominated by the T15 idiotype and encoded by the V1 gene. The IgM anti-PC antibodies so far reported in the literature have the germline S107 (T15) V1 heavy-chain V region gene re-arranged to the DFL 16.1 D and JH1 gene segments and show the least variations due to somatic mutations in their VL and VH sequences (8, 10, 15, 17). Somatic point mutations of already re-arranged and expressed VH1 region gene arose during anti-PC responses (18, 19). These V1 gene variants have a conserved site for the hapten (PC) and a variable site for the carrier.

However, a single somatically generated base substitution in the already re-arranged and expressed heavy-chain V gene can lead to a dramatic change in the specificity of this important antibody in the mouse (20). One such somatic mutant, U4, has lost the ability to bind PC and at the same time acquired the ability to bind double-stranded (ds) DNA (21).

Mutations were not detected until the second week after immunization and no mutation was found in the IgM antibodies, whereas most of the IgG and IgA antibodies had mutations (15, 17). Most of the mutated antibodies had higher affinity for antigen than their germline counterparts did (10, 17). The binding specificity of antibodies elicited by PC-KLH (keyhole limpet hemocyanin, a carrier) in BALB/c mice has shown that two distinct populations are expressed in the anti-PC memory response (22). Group I antibodies significantly express the T15 idiotype and comprise IgM, IgG3 and IgA antibodies, whereas group II antibodies bind *p*-nitrophenyl phosphocholine (NPPC) much better than they bind PC, are T15-idiotype negative and comprise most of the IgGI, IgG2a and IgG2b antibodies (23). Comparisons of mutated and unmutated T15-related antibodies showed that mutations caused a decrease in binding to *S. pneumonia*, suggesting that optimal specificity for this molecular form of PC is achieved by the germline-encoded antibodies (14). Thus, T15 may be unique in the failure of mutations to improve antigen binding (24).

In this study, we analysed PC-binding antibodies that were reactive predominantly with the PC moiety and that were of different idiotypic families, the T15 family after single amino acid substitution, the T15 allelic forms, as well as T15 with random point mutations in the CDR2 region, with the aim to find out whether activities of the PC-binding antibodies toward carrier or hapten molecules are hyearchically encoded in their VH domain. The analysis was performed using a physicomathematical approach based on the finding that the distribution of delocalized electron energies along the protein plays a crucial role in determining the protein's biological activity represented as the characteristic frequency component (25–29).

The results presented suggest that binding of the phosphate group encoded in the CDR2 region of the PC-reactive antibodies with characteristic frequency at f = 0.37 Hz is dominant. Furthermore, a peptide derived from PC-binding antibodies denoted as VH50-73 peptide from the CDR2/FR3 region with sequence complementarity (30–32) shows a dominant frequency at $f = 0.37 \pm 0.05$ Hz. However, self-association activity is only found in the VH50-73 peptide derived from the T15 proteins with $f = 0.37 \pm 0.05$ Hz, which satisfies the criterion of opposite phases for proteins that associate with each other (28, 29).

Methods

The H chain V region amino acid sequnces of the T15 hybridomas that utilize the VS107 genes and the sequence of its somatic mutant used were previously determined by Rudikoff *et al.* (20) and Perlmutter *et al.* (9), respectively; the sequences of the hybridoma proteins of C57BL and CBA/J origin and myeloma proteins M603 and M167 are from Clarke *et al.* (13) and Rudokoff and Potter (33), respectively. The T15 variants with CDR2 amino acid substitution are from Chen *et al.* (19).

The analysis of the PC-binding antibody VH sequence was performed by applying a physicomathematical model named as the informational spectrum method (ISM) and resonant recognition model (RRM). The model was described in details elsewhere (25–29). A brief description is presented here.

The basic presumption of the model is that the electron-ion interaction potential of amino acids describes the energy states of valence electrons that are important for interaction between molecules (25) and that a defined frequency domain is represented via a common frequency for proteins with the same biological function (26–29). This biological frequency is determined in several steps. In the initial step, the sequence of protein is converted into a numerical series by assigning to each amino acid the characteristic average energy of all valence electrons calculated via pseudopotential (25). Numerical series obtained in this way are treated as a signal and in the next step are transformed into the frequency domain using the Fourier transform. The sampling frequency is f_s = 1 Hz because the distance between molecules is set to d = 1. According to the Nyquist theorem, the frequencies of interest in power spectra could take values up to 0.5 Hz. In order to determine the common frequency for the two sequences, the cross-spectral function was determined according to the formula:

$$CSxy(f) = X(f)Y(f)^*; f = n\frac{f_s}{N}; n = 1,2\ldots\frac{N}{2} \qquad (1)$$

where the X, Y^* (asterisk denotes complex conjugate) are the Fourier transforms of the first and second sequences and

f denotes the frequencies of a certain discrete set of index *n* which runs to *N*/2, where *N* represents the number of points of Fourier transform. The multiple cross-spectral function of several sequences or "consensus spectrum" is calculated by the formula:

$$CS_M(f) = CS_{12}(f)CS_{23}(f).....CS_{M-1,M}(f) \quad (2)$$

where the index *M* denotes the number of sequences. If a dominant peak appears in the cross-power spectrum at a certain frequency and if it is the signal–noise ratio at this particular frequency, *S*/*N* > 20 (27–29), this frequency can be considered relevant for the biological activity. Furthermore, from the coefficients *X*(*n*) of the corresponding Fourier transformation for a particular sequence of a protein:

$$X(n) = \sum_m x(m) \cdot e^{-j\frac{2\pi f \cdot m}{f_s}} = |X(n)| \cdot e^{-j\varphi(n)}; n = 1,2...\frac{N}{2} \quad (3)$$

where index *m* counts the members of the original numerical series, the amplitude $|X(n)|$ and phase spectra $\varphi(n)$ for the characteristic frequency *f* can be determined (28, 29). Based on formula 3, it is possible to determine the phase difference $\Delta\varphi(n)$ between the phase of sequences of protein $\varphi_1(n)$ and the phase of its target $\varphi_2(n)$ at a certain frequency *f* or index *n*:

$$\Delta\varphi(n) = |\varphi 1(n) - \varphi 2(n)| \quad (4)$$

The experience based on the previous research suggests that the difference in phase between a protein and its target at a relevant biological frequency should be approximately π radians (28). In this study, the cross-spectral function was normalized and expressed as a percentage (*y*-axis) versus frequency in Hz (*x*-axis). Normalized CS was obtained by dividing its values with its maximum value and multipling by 100%.

Results

The present study was carried out to test the hypothesis of hierarchically encoded activity in the VH chain sequence of PC-binding antibodies. In order to find out whether, in the VH sequence of the PC-binding antibodies, hapten or carrier recognition was a dominantly encoded and thus represented by a characteristic frequency, a referent cross-spectral function was performed for antibodies raised against PC when the carrier was a PC-expressing strain R36a of *S. pneumoniae*. For such analysis, the prototype T15 antibody and its allelic form 1613 raised in C57BL mice were used. The common frequency component at *f* = 0.37 Hz, *S*/*N* = 34.19 is revealed (Fig. 1A). The control cross-spectral function made with antibodies that lacked specificity for PC (28) reveals a dominant common peak at *f* = 0.22 Hz, *S*/*N* = 20.81 (Fig. 1B), meaning that these antibodies and PC-binding antibodies do not have a common biological activity (Fig. 1A and B).

The PC-specific antibodies T15 and M603 are from two different idiotypic families. They do not share specificity for the carrier molecule and, in addition, antibody M603 contains amino acid replacements in the CDR2 region of the heavy-chain variable region, which indicates that CDR2 is also important in recognition of some carrier determinants. A direct implication of this finding is that CDR regions may accommodate structures larger than PC such as associated carrier determinants (34).

In order to see the impact of the mutations in CDR regions of M603 with high activity for *Proteus morganii* on the *f* = 0.37 Hz, the cross-spectral function was performed for T15 and M603 antibodies. The common dominant frequency component is still at *f* = 0.37 Hz, *S*/*N* = 20.01 (Fig. 1C), suggesting that this frequency component may characterize interaction with the hapten, i.e. PC, rather than with the carrier as PC is in the context of different carriers.

These data support the notion that the VH sequence of the PC-binding antibodies may encode interaction with hapten represented by *f* = 0.37 Hz as a dominant specificity.

With this thought in mind, we sought to examine the impact of mutations impairing antigen binding of the PC-specific T15 antibodies on the already identified common characteristic frequency *f* = 0.37 Hz that, we are assuming, delineates binding of the PC hapten.

A thorough investigation of the impact of random mutations in the H chain CDR2 of the T15 antibody on PC binding has been performed (19). It was found that majority of the analysed mutant antibodies (e.g., M22) had lost binding for the carbohydrate form of PC that is present in R36a and none of the mutated antibodies increased binding activity (19). Mutant antibody M296, which has a more than 5-fold decreased binding activity to R36a, PC protein (PC histone) and free PC compared with wild type and mutant antibody M32, which binds only to PC protein and free PC (19) were introduced into the analysis here.

We started the analysis with mutant M22 that lost antigen binding for both PC protein and R36a (19). The cross-spectral function involving T15 and its variant M22 is presented in Fig. 2A. The analysis shows the new characteristic frequency of *f* = 0.12 Hz, *S*/*N* = 8.68. Because M22 lost specificity for both hapten and carrier, the data neither confirm nor deny that *f* = 0.37 Hz defines PC binding.

Therefore, T15 and the mutated variant M32 which only binds PC protein (19) were introduced into the analysis. The cross-spectral function showed a characteristic frequency component at *f* = 0.37 Hz with *S*/*N* = 8.63 (Fig. 2B). Another T15 mutant M296 with mutations at different amino acid positions, which binds R36a and also binds the PC protein (19), was analysed and the cross-spectral function of the T15 and M296 (Fig. 2C) showed a similar spectrum to Fig. 2B. A comparison of the cross-spectrum shown in Fig. 1A with spectra obtained for mutant T15 proteins (Fig. 2A–C) reveals that mutations in the CDR2 have an impact on the spectral pattern and consequently on the *S*/*N* value and thus emphsizes a distinction between wild-type T15 H chain and variants with mutation in the CDR2 region.

However, this analysis confirmed that mutations in the M32 and M296 are tolerated in the sense that these mutants retained both slight domination of the amplitude at *f* = 0.37 Hz and an ability to bind PC even when they failed to bind the carrier molecule. Thus, at the level of the whole VH domain sequence of the PC-binding antibody and even with CDR2 saturated with point mutations by *in vitro* random mutagenesis (Fig. 2B and C), some mutations are sustainable, as a weak ability to bind PC is retained.

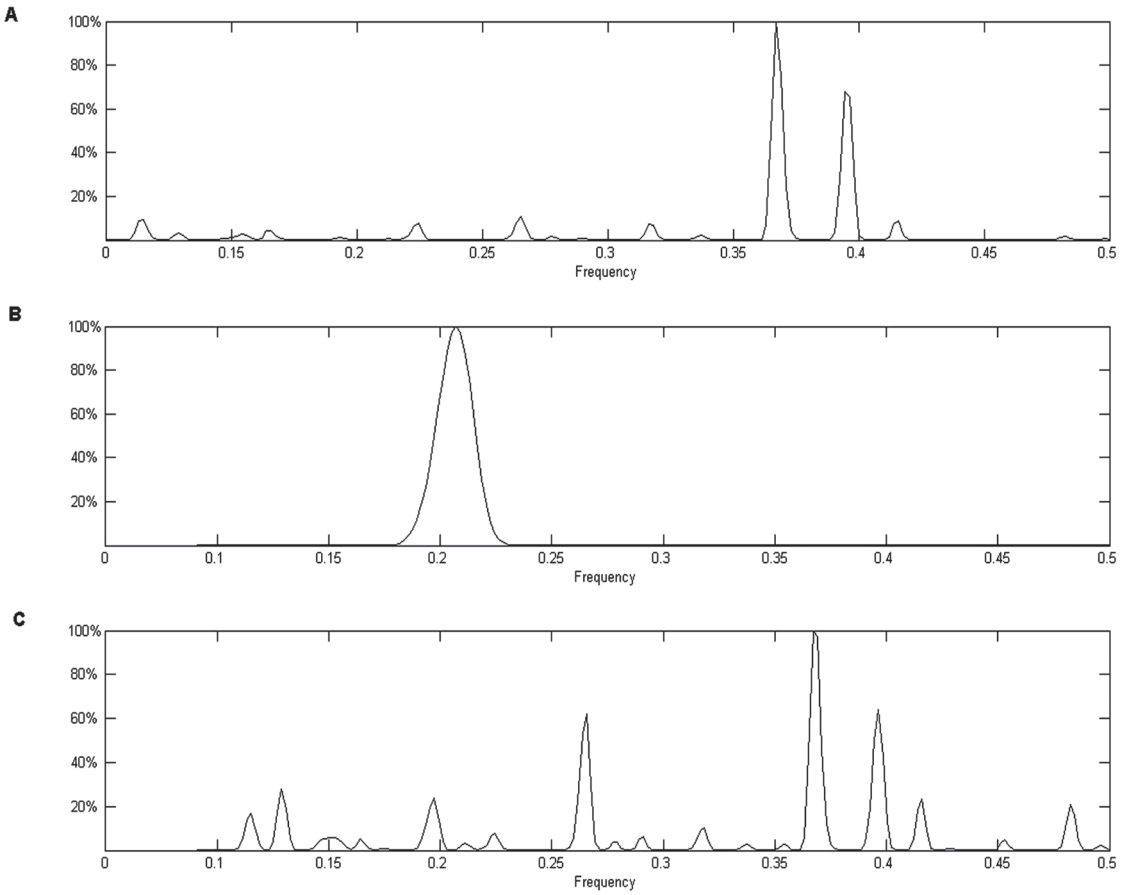

Fig. 1. Cross-spectral function of antibody heavy-chain variable region amino acid sequences. (A) Analysis of the PC-binding antibodies: T15 and 1613 antibody from C57BL mice. (B) Analysis of the non-PC-binding antibodies. (C) Analysis of the PC-binding antibodies belonging to different idiotypic families: T15 and M603.

The cross-spectral function was extended to antibodies obtained after immunization of different strains of mice using different carriers such as bacterial antigens and KLH molecules. The obtained cross-spectral function involving T15, M167, C57BL 1613, 293, 23169, and CBA/J 6F9 antibodies is presented in Fig. 2D. The obtained data confirmed that f = 0.37 Hz, S/N = 33.6 is a common frequency component for all analyzed PC-binding antibodies. Furthermore, the pattern of the spectrum is similar to the one presented in Fig. 1A.

One may argue that the conclusion about the importance of f = 0.37 Hz in the PC binding/recognition might not be correct as only mutations in the CDR2 region of the VH chain were examined and the PC binding site involves other CDR regions such as CDR1. Therefore, T15 with a somatic mutation (Glu 35 to Ala) in the VH CDR1 region was introduced into the analysis. This single mutation not only resulted in loss of binding to PC but also generated new specificity for DNA and cardiolipin (20, 21). Residues in the CDR2 form one of the three subsites for the contact with PC and interact with the dianionic phosphate group, being a crucial portion of the phosphate-binding subsite (35).

Unexpectedly, the cross-spectral function (Fig. 3A) of T15 and T15 with mutation at VH residue 35 (Glu 35 to Ala) showed an unchanged common frequency component f = 0.37 Hz but the S/N was < 20 and thus different to the spectral pattern presented in Fig. 1A. Because the self-binding or autophilic site is still present in the T15 DNA-binding mutant antibody (36), this suggests that CDR2 may take part in other important activities of PC-binding antibodies such as self-association involving the CDR2/FR3 region.

Therefore, the spectrum of the 24-mer peptide (VH50-73) derived from either T15 or the C57BL 1613 antibody that is allelic to the T15 VH of BALB/c mice (they have identical VH50-73), for example from M603 antibody with mutations in the CDR2 region, was performed in the next analysis. The data presented in Fig. 3B and C showed frequency components at f = 0.383 Hz and f = 0.385 Hz, respectively, being in the range of the method error ($f = 0.37 \pm 0.05$ Hz) with S/N < 20. It should be emphasized that the analysis is shown only for T15 mutants exibiting $f = 0.37 \pm 0.05$. The fact that the peptides retain the common frequency in the range of the method error in spite of the low S/N value may suggest that this frequency f = 0.37 Hz may define binding to the phosphate group and the ability to associate with self peptide sequences for both heavy chain and VH50-73 peptide. The self-binding phenomenon further implies complementarity between CDR2 and FR3 regions (31). If this notion is correct,

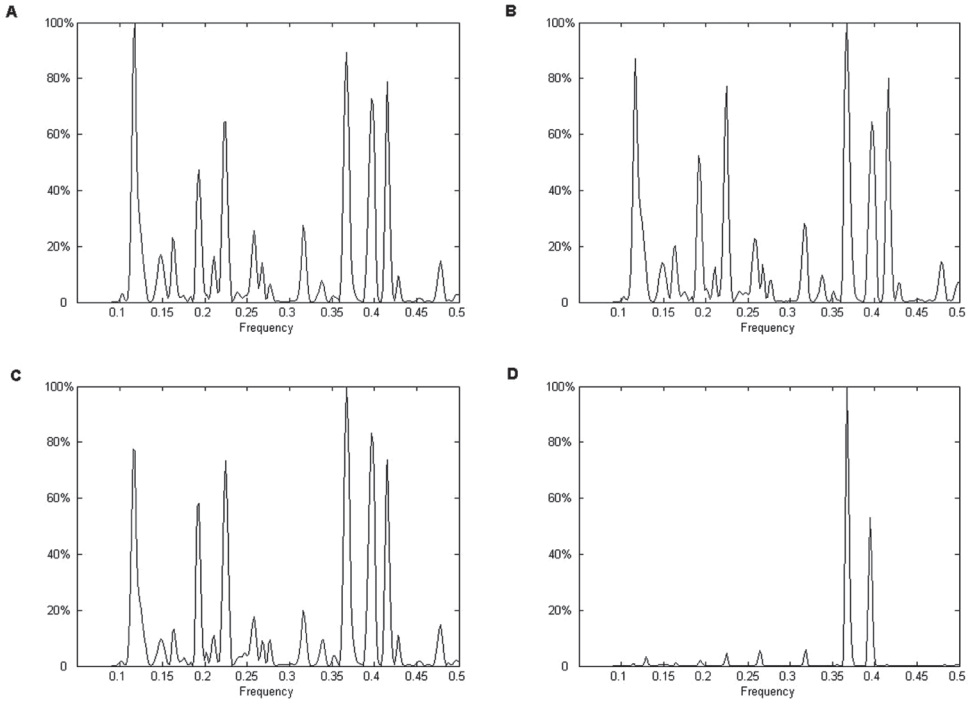

Fig. 2. Comparison of the cross-spectral function of the heavy-chain variable region amino acid sequences of the T15 antibody, T15 mutants with point mutations in the CDR2 region and PC-binding antibodies obtained in conventional immunization. (A) Analysis of the T15 and M22 mutant. (B) Analysis of the T15 and M32. (C) Analysis of T15 and M296. (D) Analysis of T15, M167, C57BL 1613, 293 and 23169, as well as CBA/J 6F9 antibodies.

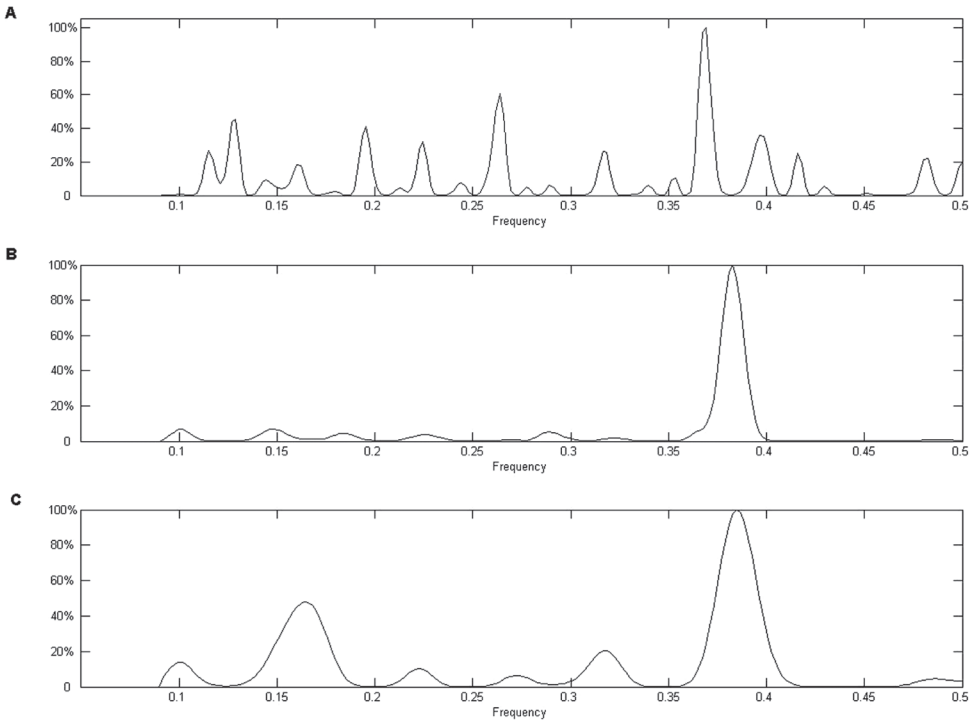

Fig. 3. Cross-spectral function of the VH regions and VH50-73 peptide sequence derived from different PC-binding antibodies. (A) Analysis of the VH region of T15 and T15 with a single mutation in VH CDR1 (Glu 35 to Ala). (B) Analysis of the VH50-73 peptide derived from the sequences of the T15 allelic forms, that is T15 and C57BL 1613 antibodies. (C) Analysis of VH50-73 peptide sequence derived from the T15 and M603 antibodies belonging to different idiotypic families' antibodies.

then one may conclude that any PC-binding antibody that contains the VH50-73 peptide and that exhibits $f = 0.37 \pm 0.05$ Hz, including those with natural mutations in the CDR2 region such as the M603 variant, is a self-binding antibody and those that do not bind PC such as M22 are not self-binding antibodies.

In order to test this presumption, we applied another criterion based on the assumption that proteins share the same characteristic frequency with their protein targets but are in the opposite phase at this frequency. In this case, the phase difference is close to $\pi = 3.14$ rad. Thus, this criterion defines the complementary sequence of interacting proteins (27, 28). The VH50-73 peptide region with previously shown hydropatic complementarities, that is VH50-60, is complementary to VH 63–74 and forms a self-association region or encode self-binding activity (31, 32). The data presented in Table 1 show that the nearest value to π was found to be 3.5 rad and is characteristic only for the VH50-73 regions derived from the T15 antibody.

Discussion

The immune response of mice to the PC moiety that is based on differential recognition by Id antibodies comprises three families (5, 6, 12, 37) of antibodies represented by myeloma proteins S107/TEPC-15 (T15), MOPC-167 (M167) and McPC-603 (M603). The VH1 gene is used to encode their VH regions (8, 9, 13–15). These antibodies have extensive homology of the heavy chains, similar affinities for the hapten PC, different affinities for its structural analogs and binding site diversity for different PC-carrier complexes. It seems that the heavy chain may well be a prerequisite for all PC-combining activity in mice.

Nuclear magnetic resonance (NMR) data (35) suggest a significant degree of conservation of important hapten-binding site interactions and support the view that the heavy chains of these proteins dominate in the interaction with bound PC while the various subspecificities of these proteins for PC analogs and the carrier itself can be accounted for by amino acid changes in the H chain hypervariable regions such as CDR2 and CDR3 and in CDR1 (20).

We assessed the phenomenon of a fixed site for PC and a variable site for carrier by postulating that binding activity for the two antigens should be hierarchically encoded in the sequence of the VH chain of the PC-specific antibodies.

The present study was carried out to test this hypothesis and the issue was approached by applying a physical and mathematical model that interprets the protein's sequence linear information using the signal analysis method of Fourier and Wavelet transforms such as the ISM or the RMM method (25–29). Previously, the method was used to investigate the periodicity of protein structural motifs (27). The dominant peak, that is the frequency component in the cross-spectral function, characterizes biological activity or interactions common for all protein sequences analyzed. In accord with this statement, we found that T15 antibody and its allelic form show a common dominant frequency (Fig. 1A), which is absent in the spectrum of antibodies that are not specific for PC (Fig. 1B).

Table 1. Correlation between phase differences at the characteristic frequencies with antibody self-binding activity

	Self-binding	Phase difference
T15	+	3.5
M22	-	1.76
M603	-	9.95

Phase differences are expressed in rad.

In nature, the PC antigen exists as the same hapten coupled to widely different carriers such as *S, pneumoniae* and *P. morganii*. An antibody response to PC in the former context was dominated by T15 antibodies, whereas the response to *P. morganii* was dominated by the M603 family (8, 13–15). Thus, between T15 and M603, anti-PC antibody variation occurs principally in carrier recognition. The data presented here reveal that T15 and M603 antibodies in their cross-spectral function have the same characteristic frequency (Fig. 1C), suggesting that the common frequency at $f = 0.37$ Hz defines PC binding activity.

The NMR analysis supported the view that the major contact residues for PC are found in the VH chain CDR1 and CDR2 of PC-specific antibodies (32 and the references therein).

However, one should keep in mind that the T15 may be unique in the failure of mutations to improve antigen binding. Therefore, to mimic effects of somatic mutation on PC binding, random mutations introduced *in vitro* by saturation mutagenesis of VH CDR2 in murine anti-PC antibody were introduced (19). Mutations in at least five different positions in VH CDR2 of the PC-specific T15 antibody can have a dramatic effect on the ability of the antibody to bind PC-containing antigens (19). Critical residues at positions Ala-50 and Arg-52 are essential for maintaining interaction between the PC and the antibody. Mutant M22 (19) that fails to bind PC has a mutation at residue 52 and loss of amplitude dominance at $f = 0.37$ Hz (Fig. 2A). However, mutations in VH CDR1 of the T15 antibody result in loss of binding to PC but also generated new specificities for DNA and phosphorylated proteins (20, 21) but the characteristic peak at $f = 0.37$ Hz was not affected (Fig. 3A).

These data forced us to investigate what other activity might be common for PC-binding antibodies in addition to their specificity for the PC and/or phosphate group represented by the shared frequency component $f = 0.37$ Hz. The answer could be a self-association or autophilic property. Furthermore, the self-binding or autophilic site is still present on T15 DNA-binding mutant antibody (36), suggesting that CDR2 may take part in another important activity of PC-binding antibodies such as self-association. Research on self-binding began with the finding of radio-labeled T15 binding to insolubilized T15, which was interpreted as showing that the T15 mimic structure of the PC antigen is important both for self-regulation and for conferring the superior protective effects of T15 (30, 32). Later on a sequence region in the T15 VH was identified as having inverse hydropathy and being as effective as PC in inhibiting the self-binding complexes (31). The part of the CDR2 of the T15 VH region extended into the FR3 region was predicted as the primary self-binding locus where the CDR2 region (VH50-60) is complementary to FR3 sequence (VH63-73) (30–32). The self-binding antibody is more potent in protecting mice against pneumoccal infection than non-self-binding antibodies and conjugation of the self-binding or autophilic peptide (T15VH50-73) to antibodies

with a different specificity is being considered as a way to enhance their therapeutic properties (32).

We argue here that subsite interaction with PC of idiotypically distinct mouse PC-binding antibodies such as T15 and M603 is represented by a characteristic frequency $f = 0.37$ Hz for the VH sequences and $f = 0.37 \pm 0.05$ Hz for the derived VH50-73 peptides (which is in the range of the method error). However, data concerning complementarity regions of the VH50-73 peptide responsible for the self-binding activity based on a suggested criterion for the opposite phase (28) reveal that self-binding is only an inherent characteristic of T15 (Table 1), that is antibodies with the CDR2/FR3 sequence ASRNKANDYTTEYSASVKGRFIVS, and this enables the protective superiority of the T15 antibodies.

Changes of amino acids in the heavy chain CDR2 may affect both $f = 0.37$ Hz (Fig. 2A) and the spectral pattern of the mutant antibodies exhibiting decreased activity toward the PC moiety (Fig. 2B and C), as well as impairing self-binding in the case of M603 with naturally occurred mutations in CDR2 region (Table 1). These data suggest that according to the criterion used in this report, the phosphate-group recognition of PC-binding antibodies is hierarchically encoded in the sequence of the heavy-chain variable region. Thus, it is tempting to speculate that self-peptides from the self-association or autophilic region of the T15 PC-binding antibody, that is antibody raised to polysaccharide epitopes, many of which are also self-antigens, in addition to being protective, may play an important immunologic role and take part in the control of autoimmunity mediated by regulatory T cells (R. Metlas and T. Srdić-Rajić - Patent application P-2011/0505 and PC/E/2012/3916, Intellectual Property Office of Serbia).

In summary, the data presented in this report suggest that according to the performed analysis, phosphate-group recognition is represented a the characteristic frequency of the PC-binding antibodies and that an ability to associate with self peptide sequences is a characteristic of the T15 PC-binding antibodies.

Funding

Ministry of Education and Science of the Republic of Serbia (171028 and III41026).

Acknowledgements

The authors would like to thank N. Rajić Cvijetić for the correction of, and advice on, English writing. The authors declare no conflicts of interest.

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